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FOLLOW-UP REPORT TO PREVIOUSLY FILED INFORMATION
INHALATION STUDY OF A POLYMER OF ACRYLIC ACID

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Enclosed is the final report of the chronic inhalation study as mentioned in the letter of July 9, 1990 from The Procter & Gamble Company for inclusion in your file.

If you have any questions, please do not hesitate to contact me.

Very truly yours,

Paul A. Wright

Paul A. Wright
Legal Department
517/636-1853

dhr

enclosure

[redacted]

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TWO-YEAR REPEATED INHALATION EXPOSURE OF F344 RATS TO MM - DISCONTINUED STUDY

FINAL REPORT

DATE: December, 1990

Submitted to: Dr. Robert C. Lindenschmidt, Divisional Toxicologist
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Submitted by: Inhalation Toxicology Research Institute
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Study Director: Dr. James D. Sun

ITRI Study No.: [redacted]
TSIN: [redacted]
DRD No.: [redacted]

Prepared for The Procter and Gamble Company under Funds-In-Agreement Number DE-F104-86AL39741 with the Lovelace Inhalation Toxicology Research Institute which is operated for the U. S. Department of Energy under DOE Contract Number DE-AC04-76EV01013.

TWO-YEAR REPEATED INHALATION EXPOSURE OF F344 RATS TO HM - DISCONTINUED STUDY

FINAL REPORT

DATE: December, 1990

Submitted to: Dr. Robert C. Lindenschmidt, Divisional Toxicologist
The Procter and Gamble Company
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Submitted by: Inhalation Toxicology Research Institute
Lovelace Biomedical and Environmental Research Institute
P. O. Box 5890
Albuquerque, NM 87185

Study Director: Dr. James D. Sun

ITRI Study No.: FY89-009
TSIN: W1433.02
DRD No.: PDSE 957

Prepared for The Procter and Gamble Company under Funds-In-Agreement Number DE-F104-86AL39741 with the Lovelace Inhalation Toxicology Research Institute, which is operated for the U. S. Department of Energy under DOE Contract Number DE-AC04-76EV01013.

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ABSTRACT

HM is a high molecular weight, hydroscopic polymer used by The Procter and Gamble Company (P&G). A large amount of this polymer is used and the potential exists for inhalation of this material during the manufacturing process. Because of this, P&G contracted the Inhalation Toxicology Research Institute (ITRI) to initiate a chronic inhalation toxicity study on HM.

The experimental plan was to expose male and female F344 rats by inhalation to target concentrations of 0.05, 0.2 and 0.8 mg HM/m^3 for 6 hr/day, 5 days/week for 104 weeks. After 6, 12 and 24 months of exposure, animals would be sacrificed and evaluated for animal survival, urinalysis, organ weight changes, and gross and histopathological lesions. In addition, the effect of HM exposure on particle clearance from lungs would also be evaluated after 6, 12, and 20 months of exposure.

Unfortunately, just prior to the 6-month sacrifice, it was discovered that the flow rates for collecting exposure chamber samples were overestimated. This error resulted in an approximately 38% overall underestimation of the actual HM exposure concentrations and it was P&G's decision to end this study after the 6 month interim evaluation time. When the measured values were corrected for the error, the actual concentrations were determined to be approximately 0.07, 0.28 and 1.08 mg HM/m^3. (These values were a mean of concentrations achieved in the male and female exposure chambers.) During this exposure period, no exposure-related clinical signs of toxicity were observed. Decreases in body weights for both male and female rats were slight and variable, with no clear dose-response relationship. At sacrifice, exposure-related gross lesions were limited to multiple, soft, white foci in the lungs and enlarged bronchial lymph nodes in rats exposed to 0.28 and 1.08 mg HM/m^3. Exposure-related increases were seen for lung weights, lung/body

weight ratios and lung/brain weight ratios for male and female rats exposed to 1.08 mg HM/m^3. Histopathological evaluations of the lungs showed pulmonary lesions characterized by centriacinar alveolitis, macrophage hyperplasia, type II epithelial cell hyperplasia, and lymphoid hyperplasia of bronchial associated lymphoid tissue, respectively, in rats exposed to 0.28 and 1.08 mg HM/m^3. These lesions generally were minimal severity in rats exposed to 0.28 mg HM/m^3, and mild to moderate severity in rats exposed to 1.08 mg HM/m^3. Lymphoid hyperplasia of bronchial lymph nodes was also restricted to rats exposed to the 0.28 (minimal to mild) or 1.08 (mild to marked) mg HM/m^3. No lung or bronchial lymph node alterations induced by exposure were microscopically evident in any rats exposed to the 0.07 mg HM/m^3 for 6 months. No other biological evaluations were performed on these 6-month sacrifice animals and all remaining animals on this study were euthanized.

Procedural changes have been made and documented so that similar sampling errors will not occur again. Because of the discontinuation of this study, it was the decision of P&G to have the ITRI initiate a new 2-year chronic inhalation study of HM. This final report details the causes for the sampling error and the corrective measures taken to prevent such errors from happening again. The in-life data, organ weights, and histopathological data from lungs and bronchial lymph nodes collected prior to the discontinuation of this study are also reported. A summary of these results are given in Table 1.

Table 1

Summary of Results - 6 Month Sacrifice of Males
Two-Year Repeated Inhalation Exposure of F344 Rats to HM - Discontinued Study

HM Exposure Concentration (mg/m^3) Mean ± SD				Clinical	Exposure-Related Gross	Exposure-Related
Target	Actual	Mortality	Terminal Body Height	Observations (incidence)	Observations (incidence or % difference from controls)	Pathological Observations (incidence)
0	0	0/10	345 ± 16	None (10/10)	None (10/10)	None (10/10)
0.05	0.076 ± 0.013	0/10	335 ± 29	None (10/10)	None (10/10)	None (10/10)
0.20	0.284 ± 0.094	0/10	332 ± 28	None (10/10)	Lung/body wt. ratio (+12%)[a]	Alveolitis (8/10) Alveolar macrophage hyperplasia (10/10) Type II epithelial cell hyperplasia (8/10) BALT hyperplasia (2/10)
0.80	1.139 ± 0.267	0/10	341 ± 16	None (10/10)	Lung wt. (+44%)[b] Lung/body wt. ratio (+46%)[b] Lung/brain wt. ratio (+42%)[b]	Alveolitis (10/10) Alveolar macrophage hyperplasia (10/10) Type II epithelial cell hyperplasia (10/10) BALT hyperplasia (10/10)

[a]Statistically different from controls at $p < 0.05$ (Dunnett's t test).
[b]Statistically different from controls at $p < 0.01$ (Dunnett's t test).

Table 1 (Continued)

Summary of Results - 6 Month Sacrifice of Females
Two-Year Repeated Inhalation Exposure of F344 Rats to HM - Discontinued Study

HM Exposure Concentration (mg/m^3) Mean ± SD				Clinical	Exposure-Related	Exposure-Related
Target	Actual	Mortality	Terminal Body Weight	Observations (Incidence)	Observations (Incidence or % difference from controls)	Pathological Observations (Incidence)
0	0	0/10	187 ± 10	None (10/10)	None (10/10)	None (10/10)
0.05	0.061 ± 0.013	0/10	185 ± 5	None (10/10)	None (10/10)	None (10/10)
0.20	0.273 ± 0.081	0/10	189 ± 9	None (10/10)	None (10/10)	Alveolitis (8/10) Alveolar macrophage hyperplasia (10/10) Type II epithelial cell hyperplasia (8/10) BALT hyperplasia (3/10)
0.80	1.022 ± 0.273	0/10	181 ± 11	None (10/10)	Lung wt. (+53%)[a] Lung/body wt. ratio (+57%)[a] Lung/brain wt. ratio (+56%)[a] Bronchial LN wt. (+150%)[a] Bronchial LN/body wt. ratio (+175%)[a] Bronchial LN/brain wt. ratio (+156%)[a]	Alveolitis (10/10) Alveolar macrophage hyperplasia (10/10) Type II epithelial cell hyperplasia (10/10) BALT hyperplasia (8/10)

[a]Statistically different from controls at $p < 0.01$ (Dunnett's t test).

I. INTRODUCTION

HM is a high molecular weight, hydroscopic polymer used by the Procter and Gamble Company (P&G). A large amount of this polymer is used and the potential exists for inhalation of this material during the manufacturing process. Therefore, P&G requires information on the potential chronic toxicity of inhaled HM.

The Inhalation Toxicology Research Institute (ITRI) has conducted a 28-day inhalation toxicity study of rats exposed to two different sources of HM. Rats were exposed to 0.2, 1.0 or 10 mg HM/m^3 (TSIN W1433.02 and W1009.02) 6 hr/day, 5 days/week for 4 weeks (20 exposure days). Some animals were sacrificed following 20 days of exposure and others were allowed to recover for 60 days before sacrifice. The results for rats sacrificed at the end of exposure showed no significant differences between exposures to W1433.02 or W1009.02 or between the sexes. There were no dose related differences in body weight in any of the exposure groups vs. the controls. However, lung weight increased in rats exposed to 1.0 (20% increase) and 10 mg HM/m^3 (100% increase) for exposures to both W1433.02 and W1009.02.

No significant lesions were seen in lungs of rats exposed to 0.2 mg HM/m^3, although scattered aggregates of alveolar macrophages were seen. A mild to moderate multifocal alveolitis was the predominant lesion at 1.0 mg HM/m^3. Moderate bronchial lymph node hyperplasia was also seen at 1.0 mg HM/m^3. At the high dose, 10 mg HM/m^3, animals showed severe pulmonary histopathology. A diffuse alveolitis, type II cell hyperplasia and lymphoid hyperplasia were evident in these rats. No histopathology associated with HM exposure was evident in trachea, larynx or nasal cavity of rats from any group. By 60 days post-exposure, centriacinar lesions in the lung completely resolved in rats

exposed to 1.0 mg HM/m^3, but rats exposed to 10 mg HM/m^3 had mild-to-moderate centriacinar lesions at 60 days after the end of exposure. No lung alterations that could be classified as HM-induced adverse effects were detected in rats exposed to 0.2 mg HM/m^3. The particle size of HM used for these studies was 1.5 to 4.0 microns (mass median aerodynamic diameter; MMAD).

For the 2-year chronic study described in this report, the primary sponsor, P&G, selected exposure concentrations of 0.05, 0.2 and 0.8 mg HM/m^3. These concentrations were based on the results from the 28-day study (20 exposure days), a toxicokinetic study, and a previous 6-month inhalation study with HM (W1009.02) not conducted at ITRI. The lowest concentration (0.05 mg HM/m^3) was chosen because it is not possible to accurately expose to concentrations of less than 0.05 mg HM/m^3 due to background dust levels in exposure chambers (about 0.01-0.02 mg particles/m^3). Exposure concentrations were chosen so that they differed by a factor of 4. The highest exposure concentration (0.8 mg HM/m^3) is about equivalent to a 1 mg HM/m^3 concentration, which produced a mild to moderate multifocal alveolitis in the 28-day inhalation study and more severe histopathology, including some pulmonary fibrosis, in the 6-month inhalation study conducted elsewhere. Since significant pathological differences were not observed between the two sources of HM, W1433.02 was selected as the test material for this study and will be subsequently referred to as "HM" in this report.

In brief, the experimental plan for the 2-year chronic study was to expose male and female F344 rats by inhalation to target concentrations of 0.05, 0.2 and 0.8 mg HM/m^3 for 6 hr/day, 5 days/week for 104 weeks. After 6, 12 and 24 months of exposure, animals would be sacrificed and evaluated for exposure-related toxic responses. Basic determinants of toxicity during the

study and at each scheduled sacrifice time included animal survival, clinical observations, body weight gain, ocular examination, clinical chemistry, hematology, urinalysis, organ weight, and gross and histopathological lesions. In addition, the effect of HM exposure on particle clearance from lungs would also be evaluated after 6, 12, and 20 months of exposure. The experimental design of these studies is summarized in Table 2 and a detailed description is given in the experimental protocol in Appendix A.

Unfortunately, just prior to the 6 month sacrifice time point, an error was discovered in the procedures used to collect exposure chamber filter samples. This error resulted in an approximately 38% overall underestimation of the actual HM exposure concentrations. Within this report, all reference to HM exposure concentrations are in terms of the actual concentrations and not to the target concentrations unless stated otherwise. Whenever data for male and female rats are presented together, aerosol concentrations are expressed as the mean concentrations achieved in the male and female chambers. Whenever data for males and females are presented separately, aerosol concentrations for the particular sex are given. Because of the sampling error, P&G decided to end this study after the 6-month sacrifices and to have the ITRI initiate a new 2-year chronic study of HM. This final report details the causes for the sampling error and the corrective measures taken to prevent such errors from happening again. The in-life data, organ weights, and histopathological data for lungs and bronchial lymph nodes collected prior to the discontinuation of this study are also reported. No other biological evaluations were performed on these 6-month sacrifice animals and all remaining animals on this study were euthanized.

Table 2

Experimental Design Summary
Two-Year Repeated Inhalation Exposure of F344 Rats to HM - Discontinued Study

Test Article	Target Concentrations[a] (mg HM/m^3)	Number of Animals[b] 6 Month Sacrifice	12 Month Sacrifice	24 Month[c] Sacrifice	Lung Retention of Surrogate Particle[d] 6, 12, 20 Months	Surveillance 6, 12, 18 and 24 Months
Air Control	0	10M/10F	10M/10F	60M/60F	8M/8F	6M/6F
HM	0.05	10M/10F	10M/10F	60M/60F	8M/8F	6M/6F
HM	0.20	10M/10F	10M/10F	60M/60F	8M/8F	6M/6F
HM	0.80	10M/10F	10M/10F	60M/60F	8M/8F	6M/6F

[a]Each treatment group was exposed to the assigned level of test compound for 6 hr per day, 5 days per week (Mon-Fri) excluding ITRI holidays that occurred during the exposure period. Exposures began on a Monday of the first week and would have ended on the Friday of the 104th. Animals remained in the chambers during nonexposure times (nights, weekends).
[b]Body weights, organ weights, ocular examination, gross necropsy, clinical chemistry, hematology and histopathology evaluations were to be done at these times.
[c]All animals remaining were to be sacrificed within 2 weeks following 104 weeks of exposure. This would have been 60 male or female animals per group minus the number that die before this time.
[d]All animals exposed to surrogate particles would not be exposed to HM on the day of surrogate particle exposure (Thursday) and day 1 following exposure (see text). These evaluations were to be made serially on the same animals at each time point.

II. MATERIALS AND METHODS

A. Chemical

1. Bulk HM

a. Name: HM

b. TSIN: W1433.02

c. ITRI Receipt Date: 6/1/88

The bulk HM obtained from P&G was a milled white powder and was characterized (identity, stability, purity) by P&G prior to shipment to ITRI. Upon receipt of HM at the ITRI, the material was repackaged in approximately 1 kg lots. Frozen standards were also prepared in which approximately 1 g aliquots were removed from the shipping container and placed in scintillation vials. The net weight was recorded to within 0.1 g. These reference standards were placed in a larger container, bagged, and stored at -70°C for periodic chemical reanalysis.

P&G was responsible for purity and stability analysis during the study. An aliquot of the HM was taken from the exposure room and shipped to P&G together with one of the frozen reference standards prior to the start of animal exposures on March 27, 1989. P&G performed the appropriate analyses to determine whether the HM was suitable for continued study. After the study was stopped on October 3, 1989, all unused chemical (approximately 13.5 kg) was returned to P&G on November 14, 1989.

The HM was stored at ITRI out of direct sunlight at room temperature. It should be noted that the relative humidity (RH) in Albuquerque, NM, where ITRI is located, is routinely less than 40%. However, the ventilation systems for the room where the bulk chemical was stored, and the exposure rooms, provide no provision for dehumidification. Thus, at times

when the ambient RH may have exceeded 40%, ITRI had no capability to reduce it. The room temperature where the HM was stored may have ranged from 50-80°F. The storage room temperature and RH was not monitored.

2. HM Aerosol Generation and Exposure System

The exposure system consisted of 8 Hazleton H2000 whole-body exposure chambers, and an aerosol generation and delivery system (Fig. 1). The H2000 whole-body exposure chambers have an approximately 2 m^3 capacity, are constructed of glass and stainless steel, and were operated at 15 ± 2 air changes per hour. HM was compacted into a Wright Dust Feeder cup at 3000 psig using a hydraulic press and the exposure aerosol was generated using Wright Dust Feeders (WDF). No other preparation of the HM powder was required. The aerosol generation system used six WDFs. Each WDF provided exposure aerosol to one chamber. Figure 2 is a schematic of the generation and delivery system for a typical layout of a chamber. The output of the WDF went through a cyclone to remove the fraction of large particles (> ~ 4 μm) from the aerosol.

All incoming diluting air to chambers and all exhaust from the exposure system passed through HEPA filters. Exposure duration was 6 hr and was initiated with startup of the generation system. The chambers remained sealed for an appropriate time period post-generation following each daily exposure to compensate for 90% aerosol equilibrium time (T90), prior to opening chambers for animal care.

Before the initial animal exposures began, satisfactory achievement of uniform targeted dust concentrations for the test material throughout the appropriate chambers using three consecutive 6-hr periods was performed and documented. This included mass and particle size distribution.

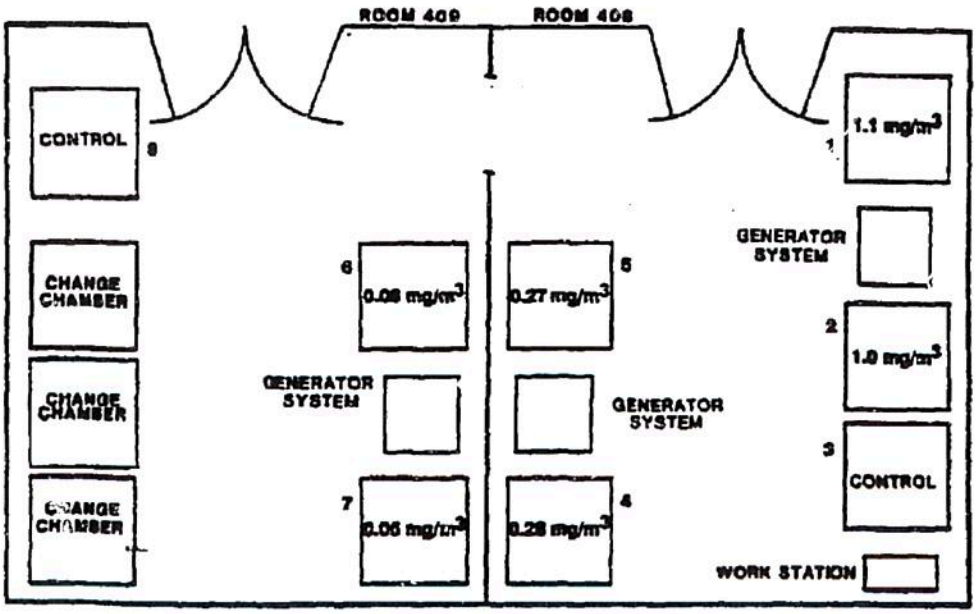
During exposures, the actual chamber concentrations were monitored daily. Samples of test material collected on 25 mm Nuclepore

Figure 1

Layout of the HM Inhalation Exposure Laboratory

Two-Year Repeated Inhalation Exposure of

F344 Rats to HM - Discontinued Study



Males = Chambers 1, 4, 6, 8 -

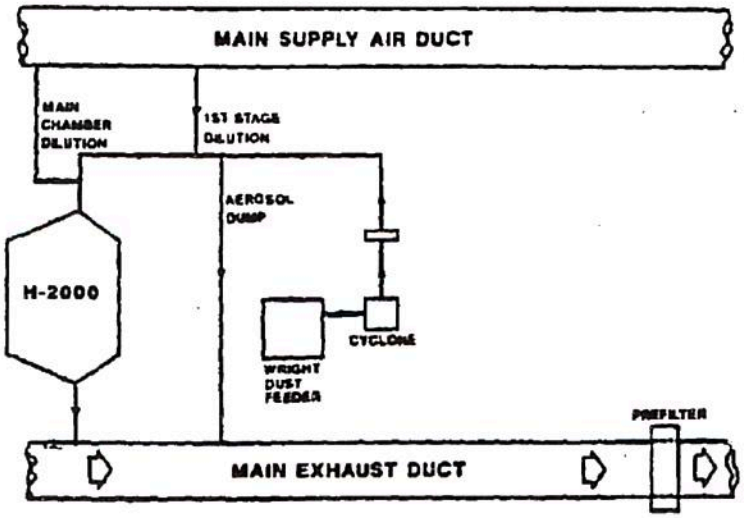
Females = Chambers 2, 3, 5, 7 -

Figure 2

Schematic of the HM Aerosol Generation and Exposure System

Two-Year Repeated Inhalation Exposure of

F344 Rats to HM - Discontinued Study



filters were weighed using a Cahn Model 30 Electrobalance (Cahn Instruments, Cerritos, CA). The amount of material collected on the filter, divided by the volume of air sampled, was used to define the chamber dust concentrations. Filter samples were taken from a fixed representative location in each chamber near the breathing zone of the animals. During the 6-hr exposure period, three 2-hr filter samples were collected at the 0.28 and 1.08 mg HM/m^3 levels. One filter was collected for the 0.07 mg HM/m^3 level and control chambers. Samples were collected on filters at the rate of approximately 3-5 liters per minute. The actual sampling rate was recorded on daily exposure forms and was used to determine actual chamber concentration.

RAM-S real-time aerosol monitors were used to observe trends in chamber concentration during the 6-hr exposures. Each chamber was monitored using the RAM-S at the start of exposure and for 5 to 10 minutes every hour during exposures. This provided the operator with a "real-time" indicator of the relative chamber concentrations and provided the basis for decisions relative to adjustments to the exposure system.

An aerodynamic particle sizer, the Aerosizer Mach 2 (Amherst Process Instruments, Inc., Amherst, MA), was the primary instrument used to measure aerosol size distribution at each aerosol concentration. Samples were obtained daily from each chamber during the prestudy phase and the first month of the chronic exposure and weekly thereafter. Cascade impactor samples were used to verify results of the aerosizer for the 1.08 mg HM/m^3 aerosol only. Based on the results of the 28-day HM study, it was assumed that the aerosol size distributions for all exposure levels are approximately the same. Therefore, impactor samples were not taken from the 0.28 and 0.07 mg HM/m^3 exposure chambers. Aerosol size distribution data (mass median aerodynamic

diameter and geometric standard deviation) were determined by a cascade impactor during the prestudy phase and weekly during the first month of the chronic exposure for the 1.08 mg HM/m^3 chambers. Impactor samples were taken monthly thereafter on the 1.08 mg HM/m^3 chamber.

Aerosol rise and fall time (T90) were determined during the prestudy phase (without animals) and verified once for each chamber during the first two weeks of the chronic exposure (with animals).

Chamber temporal and spatial variation of aerosol mass concentration were determined in each chamber during the prestudy phase (without animals) and repeated once for each chamber during the first two weeks of the chronic exposure (with animals). Variation determinations were done on each chamber every 3 months thereafter.

3. Chemical Analysis

Chemical analysis of HM was performed based on the sodium (Na) content of the sample. Selected filters, used for HM gravimetric determination, were used for these Na analyses. These HM samples were collected from each chamber on pre-weighed membrane filters (Nuclepore filters) and re-weighed to determine gravimetric concentration. The filters were then placed in vials with 0.5 mL isopropanol and 9.5 mL 1:100 ionic strength adjustor (1:100 ISA) (0.04 M NH_4Cl/0.04 M NH_4OH) to dissolve the HM. This solution was analyzed for sodium content with a sodium specific ion electrode. With each set of samples analyzed there were samples spiked with NaCl to determine the recovery of Na, and samples of HM to determine the % Na in HM. (The % Na in HM was determined from a sample of HM stored in a dessicator and dried overnight at 100°C prior to weighing.) The mass of HM on experimental samples was calculated using the Na measured and the experimentally determined % Na in HM.

Analyses were performed on all 280 filters collected during the first 4 weeks of exposure for each sex. However, analyses of the filters collected monthly were not completed by the time the study was terminated, because analyses were proceeding behind schedule. No analyses were performed on filters collected after May 1989. However, there is no reason to expect a change on the Na content of the HM aerosol.

B. Animals and Animal Maintenance

A total of approximately 430M/430F healthy appearing [CDF® (F344)/Crl Br; VAF/Plus™] rats, 26 days old when received from Charles River Laboratories (Kingston, NY), were required for this inhalation study. A total of 376M/376F rats were placed on study (Table 1). Female rats were received 2 weeks after male rats. Start of exposure for females was 2 weeks later than males so that all animals could be sacrificed within 2 weeks after the end of exposures. Female rats were exposed to HM in different chambers than those used for male rats.

The animals were quarantined in H2000 inhalation chambers for 20 days prior to start of the exposure. All rats received at the Institute for the HM study received a gross ophthalmic examination using a penlight by a veterinarian to determine if any of the animals had visible ophthalmic lesions. At the end of the quarantine period, animals of questionable health (including those documented to have exhibited any of the following signs: wheezing, nasal discharge, shallow/irregular respiration, alopecia, palpable/visible growths, listlessness, ataxia, hypothermia, rough furcoat, arched spine, excess salivation, dull/opaque eyes and diarrhea), or outlying body weight (greater or less than 25% of the median body weight for the sex) were excluded from the study.

1. Animal Randomization

Animals were randomly assigned to each exposure group by sex and body weight determined as outlined in Table 2 using the MicroVAX computer and the Path/Tox software. Before the first day of exposure, animals within each exposure group were randomly assigned using the RS1 software randomization procedure to the 6, 12, or 24 month sacrifice or to the "lung retention of surrogate particle" or "surveillance" groups (see Table 1). All animals received an identification letter designating the exposure group and a unique animal number which was used for the tail tattoo. Animals not placed on study were euthanized.

2. Animal Disease Screening Program

a. Prior to Exposure

Within 48 hr of arrival, animals were examined by a veterinarian to determine their health status. Approximately 3-4 days prior to the first day of exposure, animals were re-examined by a veterinarian and five male and five female rats were selected for health screening. Blood for serological testing was collected and tested for antibodies for Sendai, PVM, RCV/SDAV, KRV, H-1, Reo Type 3, LCM, M. pulmonis, and CAR bacillus. All serological testing was done by Microbiological Associates (MBA; 9900 Blackwell Rd., Rockville, MD 20850). Each surveillance animal received a complete necropsy. Lungs, trachea, heart, liver, kidney, stomach, small and large intestine, spleen, brain, Harderian gland, salivary glands, eyes, nose and organs with gross lesions were retained in 10% neutral buffered formalin.

b. During Exposure

Six male or six female rats were designated as sentinels in each of the eight exposure chambers, for a total of 24 sentinels per sex.

Blood was obtained by retro-orbital bleeding during halothane anesthesia from one male or one female sentinel from each chamber after 6 months of exposure. The sera were tested for antibodies by MBA for the pathogens described above. The additional sentinels in each chamber were to be tested or used for pathological evaluation should evidence of disease have been found.

c. Animal Housing

Animals were individually housed in stainless steel wire mesh cages (5 11/16 x 11 x 8 inches for male and 3 13/16 x 11 x 8 inches for female rats) within the H2000 inhalation chambers during inhalation exposures. Cage maps were prepared showing the specific location of animals within the exposure chamber. The cage racks were rotated clockwise weekly during the chamber changeout procedure. The environmental conditions of the exposure chamber and exposure room met the following specifications:

1) The light/dark cycle was 12 hr light - 12 hr dark, with light starting at about 0600 AM.

2) The temperature of the room was maintained to provide proper range of temperature in the exposure chambers as close as possible to 75 ± 3°F (range).

3) The relative humidity was maintained as close as possible to 40-70%.

4) The flow rate of air through the chamber was maintained as close as possible to 15 ± 2 air changes/hour.

Chamber environmental conditions (flow, pressure, temperature, and relative humidity) were monitored by a real-time computer-based monitoring system. Data collected by the monitoring system were archived daily in the main computer system. Data included the mean, standard deviation, minimum and maximum values, and any out-of-limit values.

d. Diet and Water

All animals were fed certified Wayne Lab Blox (Allied Mills, Chicago, IL) and water ad libitum throughout the study except during the inhalation exposure when feed was withheld. During exposure, only water delivered by automatic watering systems was available. A representative sample of the animal drinking water at the ITRI is analyzed for contaminants at least once each year.

C. Biological Measurements and Observations

1. Clinical Observations and Body Weights

Test animals were observed for signs of toxicity, morbidity, and mortality twice per day, 7 days per week, including holidays. Each rat was examined for clinical signs prior to start of exposure, weekly during the first 13 weeks, and at 4 week intervals thereafter. These observations were recorded utilizing the Path/Tox computer system. Abnormal clinical signs detected at times other than the weekly or monthly observations were noted and recorded in the Animal Room Log.

Body weights were determined for all test animals prior to exposure, weekly for the first 13 weeks, and at 4 week intervals thereafter, using the Path/Tox computer system (Xybion Medical Systems, Cedar Knolls, NJ).

Clearance of a surrogate particle was to be measured in 8 male and 8 female rats from each exposure group at 6, 12 and 20 months of exposure. The same animals were to be used at each time point. This would have determined if exposure to HM alters long-term lung clearance mechanisms. This evaluation was not performed.

2. Sacrifice and Necropsy

Approximately one week prior to the 6-month sacrifice, animals were assigned to a provisional order of sacrifice according to the procedures

described in the protocol. Feed was not given to the rats the night prior to their scheduled necropsy. At necropsy, animals were weighed and then anesthetized with halothane. After the animal was anesthetized and prior to exsanguination and necropsy, an ocular examination was performed by the attending pathologist to detect corneal or lenticular opacities. Any detectable corneal or lenticular alterations was recorded on the necropsy form.

The following organ weights were measured to the nearest 0.001 g on scheduled sacrifice animals: lungs, liver, kidney (pair), brain, spleen, heart (excluding major vessels), testes (males only), adrenals, thymus, bronchial lymph nodes and ovaries (females only). No organ weights were determined on animals found dead or moribund.

At necropsy, the tissues listed in the protocol were examined and removed from each animal and fixed in a 10% neutral buffered formalin solution (10% NBF) at a volume dilution of 1 part tissue to at least 10 parts 10% NBF. Histopathological examinations were done only on lungs and bronchial lymph nodes.

3. Clinical Pathology and Urinalysis

Hematologic and clinical chemistry evaluations were to be conducted on 10 male and 10 female rats/exposure group and control group at 6 months of exposure. Blood samples were collected from male animals scheduled for necropsy during exsanguination by heart puncture and placed into collection tubes containing EDTA anticoagulant for hematological evaluation and in tubes without anticoagulant for clinical chemistry evaluations. The hematological parameters that were to be determined were as stated in the protocol. However, because of the discontinuation of this study, these evaluations were not performed or the data were incomplete and therefore not reported. These data will be obtained from the restarted chronic study.

Urinalysis was to be conducted on 10 male and 10 female rats/ group prior to sacrifice at 6 months. These animals were placed in metabolism cages for an approximate 16-hr overnight urine collection. The parameters to be determined were as specified in the protocol. Because of the discontinuation of this study, these data were incomplete for male rats and similar evaluations
were not initiated for female rats, and therefore the limited data obtained is not reported. These data will be obtained from the restarted chronic study.

D. Statistical Analysis of Data

Body weight data for all animals on study and the terminal body weight for the 6-month scheduled sacrifice animals were statistically analyzed separately for each sex and for each exposure group by Dunnett's t test. Absolute organ weights, organ/body weight ratios, and organ/brain weight ratios for the 6-month scheduled sacrifice animals were statistically analyzed separately for each sex and for each exposure group by t tests. Dunnett's t tests was used if the data were homogeneously distributed by Bartlett's test. A modified t test was used if the data were not homogeneously distributed by Bartlett's test.

III. RESULTS

A. HM Aerosol Characterization and Exposure System Operation

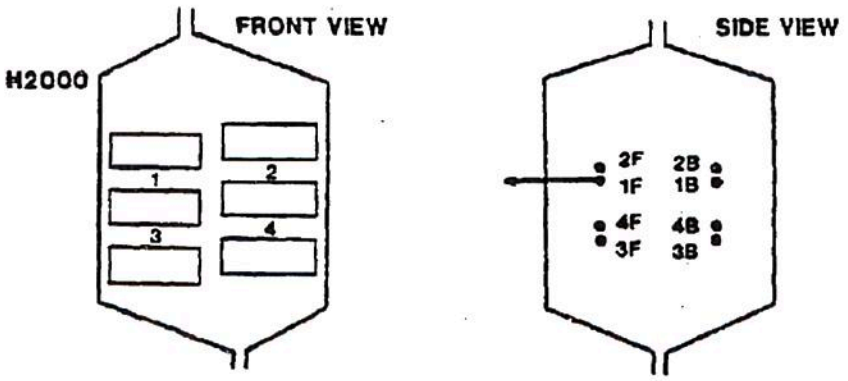
The variability (chamber homogeneity) of the HM aerosol within each exposure chamber was measured with the RAM-S unit in a sequential procedure. Homogeneity determinations were made during the start of the study and every three months thereafter. The eight sampling positions for the H2000 chamber shown in Figure 3 were used. The reference point was position 1B. Three

Figure 3

Location of Exposure Chamber Sampling Positions

Two-Year Repeated Inhalation Exposure of

F344 Rats to HN - Discontinued Study



samples were taken at the reference point at the start, middle, and end of the procedure. One sample each was taken from the other locations in between the reference samples. The total variation of aerosol concentration is the coefficient of variation of samples taken at the eight different locations and the temporal variation is the coefficient of variation of the three reference samples. Spatial variation can be calculated using the following equation:

$$CV^2_{total} = CV^2_{spatial} + CV^2_{temporal},$$

where CV (coefficient of variation) is defined as 100% times the standard deviation of the measurements divided by the mean of the measurements.

Results of the homogeneity measurements, which include the %CV for total, spatial, and temporal variation data for the study are summarized in Table 3. More detailed results are given in Appendix B, Table B-1.

The API, Inc. Aerosizer Mach 2 aerodynamic particle sizer was used to measure particle size distributions in all exposure chambers. Data collected prior to 11/17/89 used an incorrect value for the density of HM, and as a result the Aerosizer size distribution measurements are not reported.

Aerosol particle size of the high level (1.08 mg HM/m^3) chambers was also determined using a Lovelace multijet cascade impactor at a nominal flowrate of 15 L/min. Probit analyses of the mass collected on each stage of the impactor and the associated particle size range represented by that mass was used to determine the mass weighted aerodynamic diameter and geometric standard deviation of the aerosol sampled. Table 4 summarizes the size data. Although impactor data were not obtained from the 0.28 and 0.07 mg HM/m^3 levels, it is reasonable to assume that the size distributions for all

Table 3

Distribution of HM Aerosol
Two-Year Repeated Inhalation Exposure of
F344 Rats to HM - Discontinued Study

Chamber No./ HM Exposure Concentration (mg/m^3)	Date	Temporal %CV	Spatial %CV	Total %CV
1/1.14 (Males)	4/5/89	13.06	3.79[a]	3.79
	6/20/89	5.45	3.45[a]	3.45
	9/12/89	3.30	4.71	5.75
2/1.02 (Females)	4/14/89	3.47	5.13	6.19
	6/21/89	0.90	6.67	6.73
	9/18/89	5.49	3.23[a]	3.23
4/0.28 (Males)	3/29/89	1.25	4.37	4.55
	6/22/89	10.99	8.51[a]	8.51
	9/13/89	10.29	6.67[a]	6.67
5/0.27 (Females)	4/13/89	1.59	1.99	2.55
	6/23/89	0.97	17.93	17.96
	9/19/89	3.00	4.36	5.29
6/0.08 (Males)	3/28/89	1.79	5.15	5.45
	6/26/89	1.28	5.10	5.26
	9/14/89	5.00	0.00	5.00
7/0.06 (Females)	4/12/89	2.78	2.95	4.05
	6/27/89	5.95	11.36	12.82
	9/20/89	1.59	6.36	6.56

[a]Since the reference (temporal) %CV is greater than the total %CV, the equation used to calculate spatial %CV does not apply. The value of spatial %CV is approximated by total %CV.

Table 4

Aerosol Size Distribution Using the Lovelace Multi-Jet Cascade Impactor
1.08 mg HM/m^3 Chambers (CH1 = Males, CH2 = Females)
Two-Year Repeated Inhalation Exposure of
F344 Rats to HM - Discontinued Study

Chamber Number	Date Sampled	MMAD (microns)	GSD
1	3/28/89	2.68	2.07
1	4/3/89	3.18	2.06
1	4/12/89	3.06	2.21
1	4/18/89	2.80	2.40
1	4/24/89	2.62	1.82
1	5/22/89	3.84	2.73
1	6/19/89	4.94	2.93
1	7/17/89	2.57	1.90
1	8/14/89	3.02	1.99
1	9/11/89	4.22	2.29
2	4/12/89	2.40	2.28
2	4/18/89	3.70	2.20
2	4/25/89	1.85	1.87
2	5/22/89	2.38	1.87
2	6/19/89	3.32	2.34
2	7/17/89	1.83	1.46
2	8/14/89	3.52	1.92
2	9/11/89	2.62	2.48

exposure chambers were similar because identical aerosol generation systems were used for all exposure chambers. In addition, results from the 28-day HM study show that particle size distribution was not significantly different for the 0.2 and 1.0 mg HM/m^3 exposure levels.

Male F344/N rats were exposed from 3/27/89 to 10/3/89 and female F344/N rats were exposed from 4/10/89 to 10/3/89. Exposures were discontinued on 10/3/89 as a result of actual exposure concentrations being approximately 38% above original target concentrations of 0.05, 0.2, and 0.8 mg HM/m^3. The details of this event are as follows.

On Monday, September 11, 1989, a backup operator of the HM exposure system discovered an error in the procedures used to collect chamber filter samples. This situation was immediately reported to the ITRI management. Discussions with the primary operator of the HM exposure system on Tuesday, September 12, 1989 verified that he had been using the incorrect procedure since the study start of 3/27/89. He was setting the filter sample rotameters to the indicated flowrate and then connecting the sampler to the sample line. The sampler should have been connected to the sample line prior to adjusting the rotameter since the large pressure drop across the Nuclepore filters causes a significant reduction of flow through the rotameter. This procedural error resulted in an overestimation of the flowrate for all samples taken to date for the study.

As a result of the overestimation of sample flowrates, the actual chamber concentrations during the first six months of the study were significantly higher than the values recorded on the daily exposure sheets. In order to correct the recorded values, the sample flowrates were corrected to actual values. We were able to accurately estimate (within ± 3%) the

actual flowrates from the pressure drop measurements recorded for each filter sample. Flowrate as a function of pressure drop was determined for each rotameter (Figs. 4-11), because, while flowrate varies linearly with pressure drop, the slope of the line is not the same for all rotameters. A linear equation was used to correct all sample flow rates for each rotameter, and filter concentrations were corrected based on these new flowrate values. Table 5 summarizes the initially recorded and corrected concentration data for the entire exposure period (through 10/3/89). The procedures used to make aerosol concentration corrections are given in Appendix B, Table B-2. Also given in Appendix B are the weekly uncorrected aerosol concentration data with the sample filter pressure drops and flow rate data (Table B-3), the weekly uncorrected aerosol concentrations with correction factors and corrected concentrations (Table B-4), and a weekly summary of chamber aerosol concentrations corrected for filter sample flow rates and control chamber background levels of aerosol (Table B-5).

The following corrective actions were taken to prevent such an error from happening again. The ITRI SOPs for rotameters and filters (ITRI SOP No. 0439, "Calibration and Operation of Rotameters", and ITRI SOP No. 0230, "Operation of Filters") were reviewed and found that they did not include sufficient details for step-by-step instructions to correct sample flowrates when using rotameters. These SOPs were revised to include a more comprehensive explanation of the procedures to be followed when collecting aerosol samples. The revised SOPs were then discussed with all Chronic Exposure Section (CES) technicians by the CES supervisor and the aerosol scientist for the HM study.

The ITRI SOP for the operation of the HM chronic exposure system (ITRI SOP No. 0841, "Operation of Inhalation Exposure Systems - Project 5640: Two Year Repeated Inhalation Exposure of F344 Rats to HM") was also reviewed

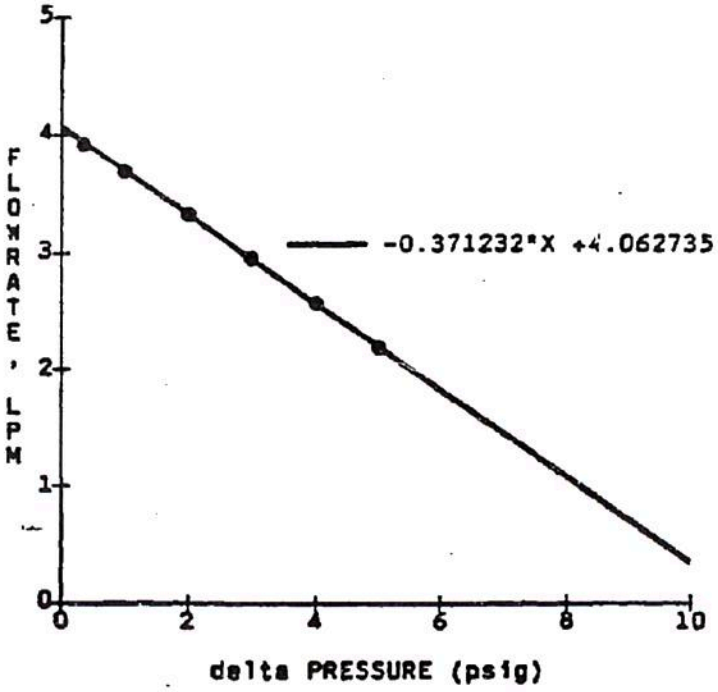
Figure 4

Sample Rotameter Flow Rate vs. Pressure Drop

for the 0 mg HM/m^3 Chamber (#8) - Male Rats

Two-Year Repeated Inhalation Exposure of

F344 Rats to HM - Discontinued Study



[Note: rotameter setting was 96 at 0 delta P.]

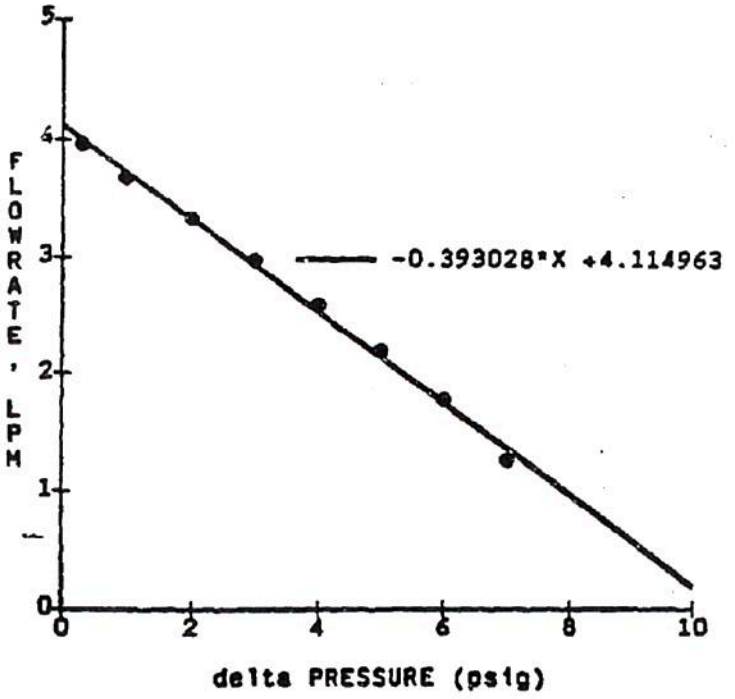
Figure 5

Sample Rotameter Flow Rate vs. Pressure Drop

for the 0.08 mg HM/m^3 Chamber (#6) - Male Rats

Two-Year Repeated Inhalation Exposure of

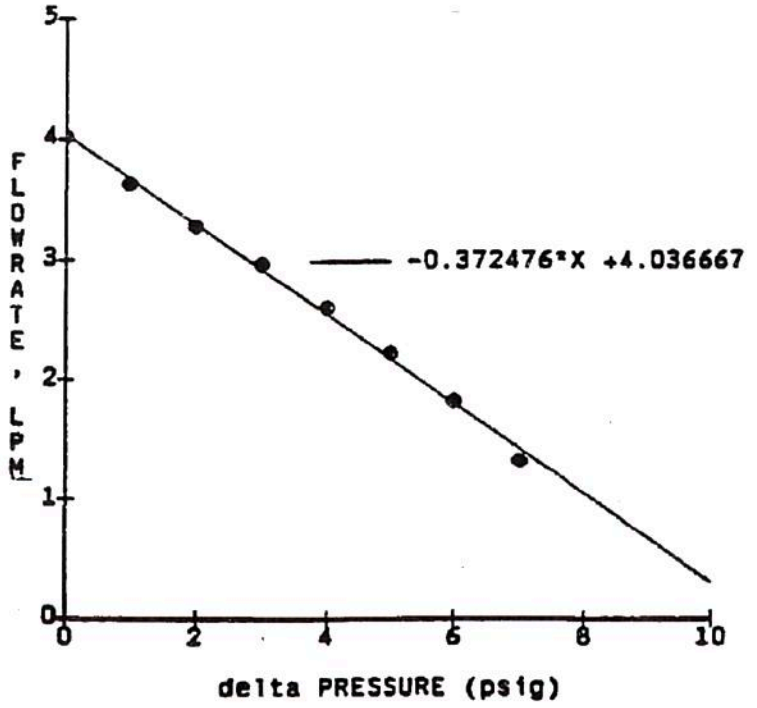
F344 Rats to HM - Discontinued Study



[Note: rotameter setting was 96 at 0 delta P.]

Figure 6

Sample Rotameter Flow Rate vs. Pressure Drop
for the 0.28 mg HM/m^3 Chamber (#4) - Male Rats
Two-Year Repeated Inhalation Exposure of
F344 Rats to HM - Discontinued Study



[Note: rotameter setting was 98 at 0 delta P.]

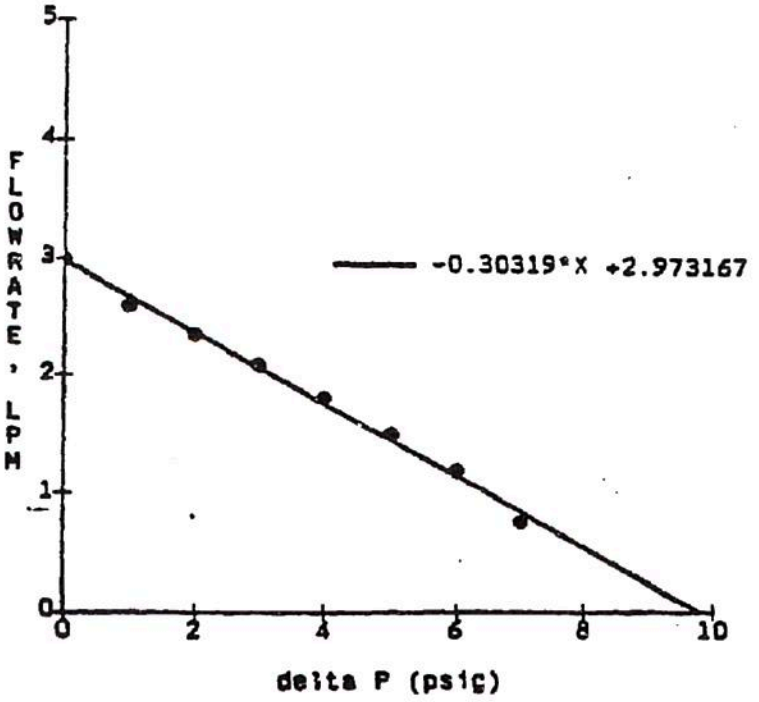
Figure 7

Sample Rotameter Flow Rate vs. Pressure Drop

for the 1.14 mg HM/m^3 Chamber (#1) - Male Rats

Two-Year Repeated Inhalation Exposure of

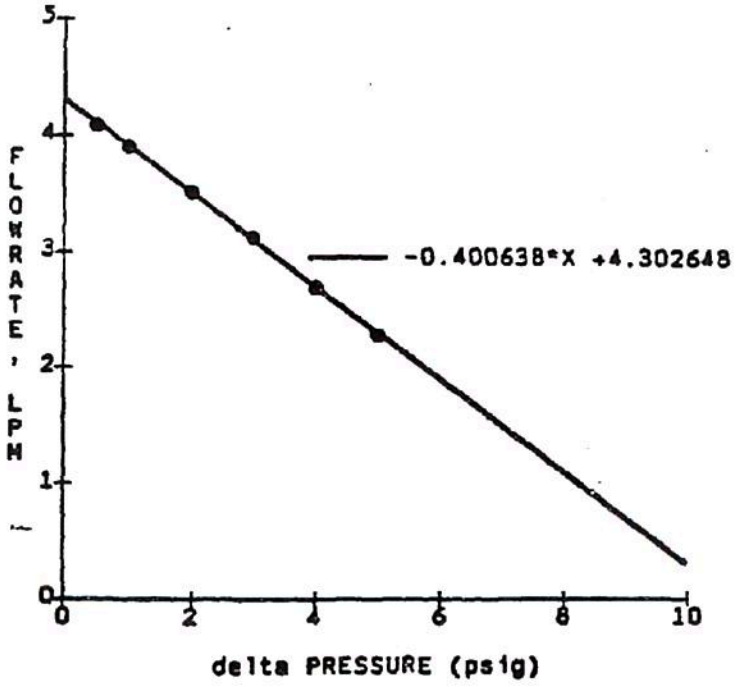
F344 Rats to HM - Discontinued Study



[Note: rotameter setting was 68 at 0 delta P.]

Figure 8

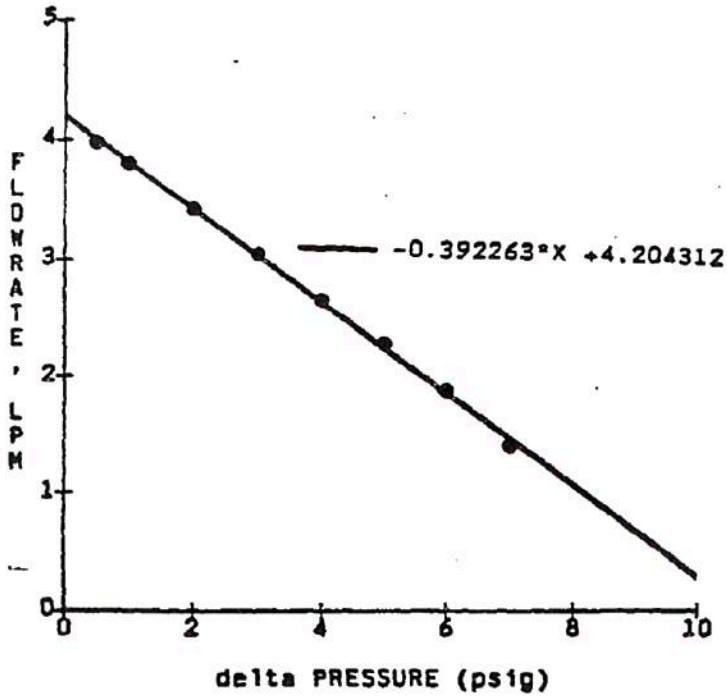
Sample Rotameter Flow Rate vs. Pressure Drop
for the 0 mg HM/m^3 Chamber (#3) - Female Rats
Two-Year Repeated Inhalation Exposure of
F344 Rats to HM - Discontinued Study



[Note: rotameter setting was 98 at 0 delta P.]

Figure 9

Sample Rotameter Flow Rate vs. Pressure Drop
for the 0.06 mg HM/m^3 Chamber (#7) - Female Rats
Two-Year Repeated Inhalation Exposure of
F344 Rats to HM - Discontinued Study



[Notes: rotameter setting was 99 at 0 delta P.]

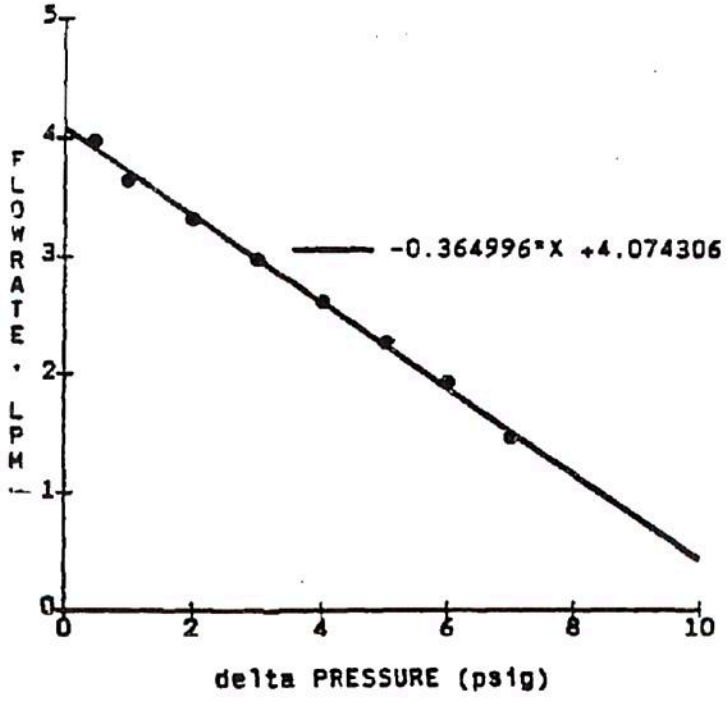
Figure 10

Sample Rotameter Flow Rate vs. Pressure Drop

for the 0.27 mg HM/m^3 Chamber (#5) - Female Rats

Two-Year Repeated Inhalation Exposure of

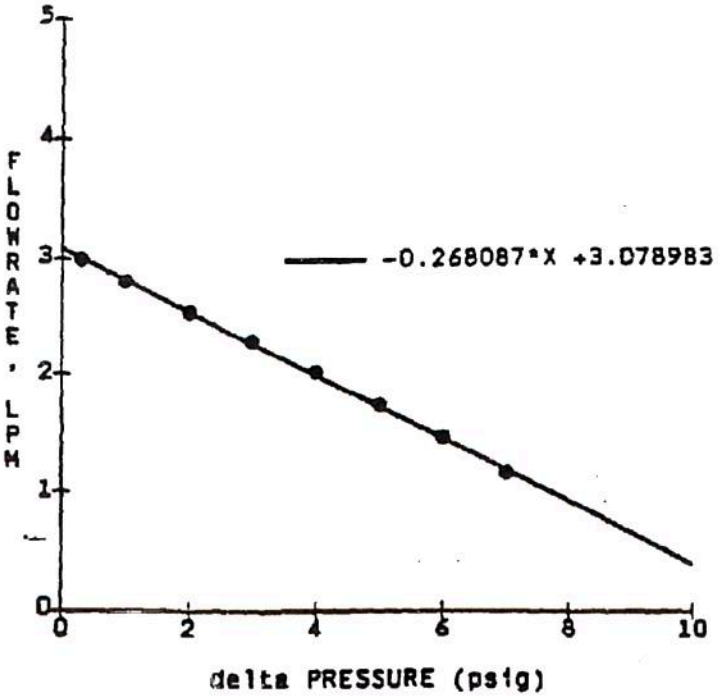
F344 Rats to HM - Discontinued Study



[Note: rotameter setting was 97 at 0 delta P.]

Figure 11

Sample Rotameter Flow Rate vs. Pressure Drop
for the 1.02 mg HM/m^3 Chamber (#2) - Female Rats
Two-Year Repeated Inhalation Exposure of
F344 Rats to HM - Discontinued Study



[Note: rotameter setting was 69 at 0 delta P.]

Table 5

Summary of HM Concentration Data[a]
3/27/89 Through 10/3/89
Two-Year Repeated Inhalation Exposure of
F344 Rats to HM - Discontinued Study

Chamber No./ Target HM Concentration (mg/m^3)	Recorded HM Concentration[b] (mg/m^3) (Mean ± SD)	Corrected HM Concentration[c] (mg/m^3) (Mean ± SD)
1/0.8 (Males)	0.781 ± 0.172	1.139 ± 0.267
2/0.8 (Females)	0.776 ± 0.194	1.022 ± 0.273
4/0.2 (Males)	0.201 ± 0.070	0.284 ± 0.094
5/0.2 (Females)	0.198 ± 0.061	0.273 ± 0.081
6/0.05 (Males)	0.053 ± 0.009	0.076 ± 0.013
7/0.05 (Females)	0.046 ± 0.010	0.061 ± 0.013

[a]All values corrected for control chamber concentrations.
[b]The recorded concentrations are the values reported using the incorrect flowrates.
[c]The corrected concentrations are the original recorded values times a correction factor (the ratio of the original recorded flow rate divided by estimated actual flowrate. See Appendix B, Table B-2).

and revised to include sections to detail sampling procedures which were not adequately detailed in the previous version. The revised SOP was then reviewed with the primary and backup operators of the HM exposure system.

All CES technicians attended a workshop in use and calibration of rotameters, critical orifices, orifice meters, impactors, filter samplers, and bubble flow meters. This workshop provided technicians an opportunity to work with these instruments under the direct supervision of the CES supervisor and the aerosol scientist for the HM study. This training was documented and included in the training file of the technicians.

B. HM Chemical Analysis

The average percentage of Na from weekly analysis of bulk HM that had been dried overnight at 100°C was 15.3 ± 1.5% (SD; n = 21). Recovery of NaCl spikes from blank filters to validate these analyses averaged 98.8 ± 5.0% (SD; n = 63). Using these data and procedures, the HM mass on randomly selected filter samples as measured by Na analysis was compared to the gravimetric determinations. Mass determinations made gravimetrically were 32.5 ± 0.2% (mean ± SD; n = 295) higher than those measured by Na analysis. The higher mass measurements determined gravimetrically were probably due to the water absorbed by the HM aerosol in the exposure atmosphere and on the filter during sample collection. Detailed data regarding these chemical analyses of HM is given in Appendix C.

C. Body Heights and Body Height Gains

Mean group weights of all rats on study obtained prior to study start (for randomization) and weekly for the first 13 weeks and monthly thereafter are shown in Figures 12 and 13, for male and female rats, respectively. Group mean body weights on day 1 and day 175 (approximately

Figure 12

Mean Body Weights of Male F344 Rats

Two-Year Repeated Inhalation Exposure of

F344 Rats to HM - Discontinued Study

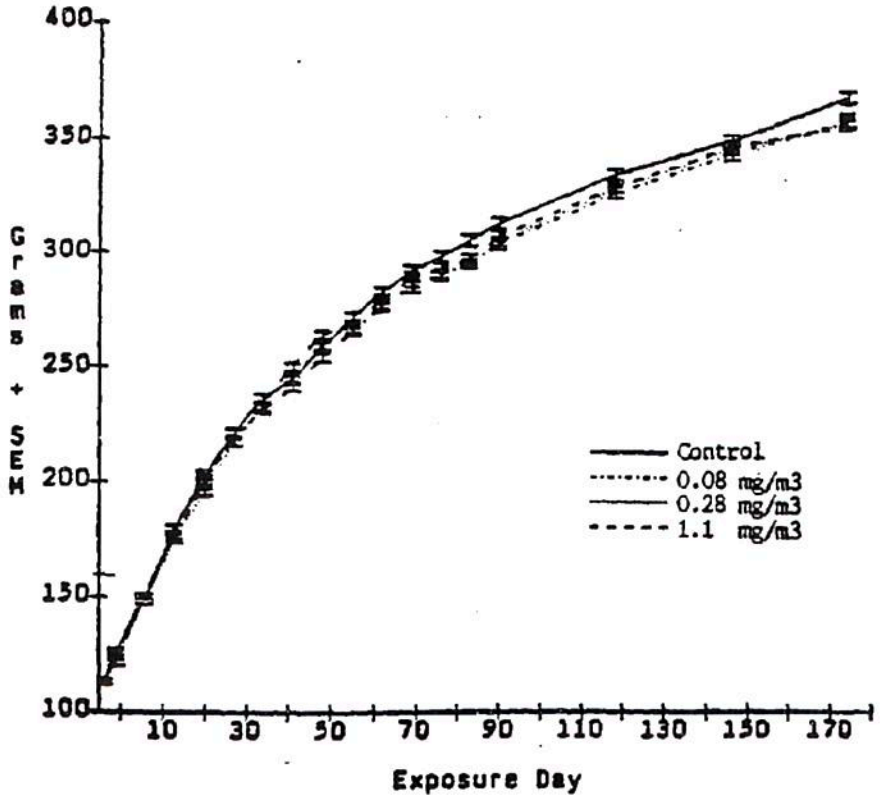
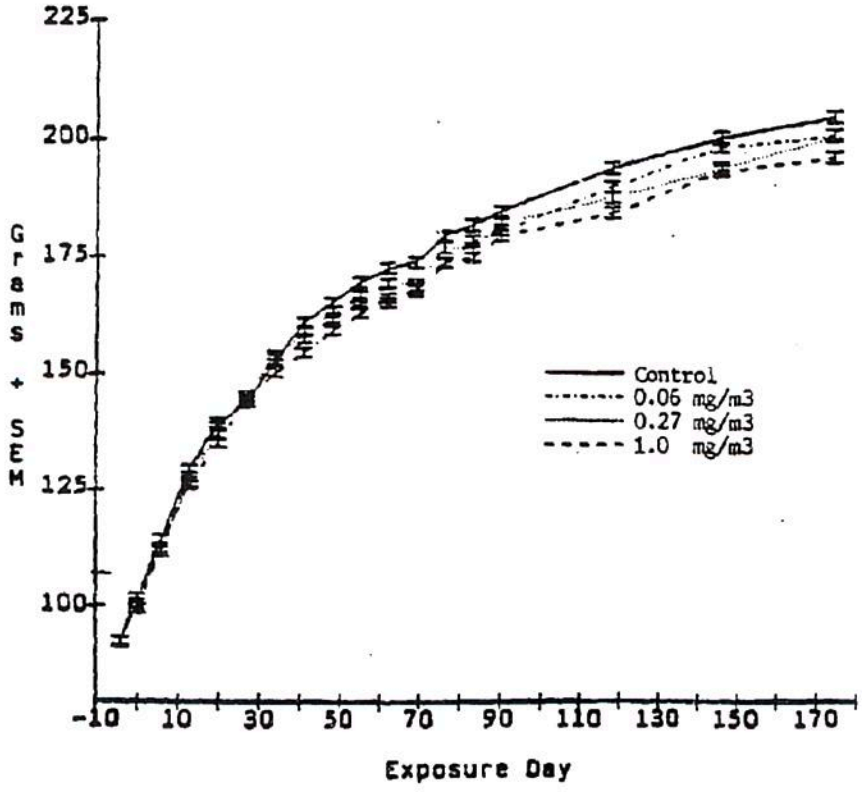


Figure 13

Mean Body Weights of Female F344 Rats

Two-Year Repeated Inhalation Exposure of

F344 Rats to HM - Discontinued Study



6 months) on study and group mean weight gain over the exposure period for all rats are given in Table 6. It can be concluded that the difference in body weights of HM-exposed male and female rats compared to control rats was slight and variable with no clear dose-response relationship. Individual in-life body weights are given in Appendix D, Table D-1.

D. Mortality

No exposure-related deaths of male or female rats occurred during this study.

E. Clinical Observations

No clinical signs of toxicity that could be attributed to HM exposure were found during the daily or detailed observation times. The incidence of discharges from the eyes was notably higher in female compared to male rats. However, because the incidence was similar among exposed and control rats, we feel that the occurrence of the eye discharge is not related to HM exposure. Clinical signs, determined weekly, are summarized in Appendix D, Table D-2.

F. Necropsy

1. Gross Necropsy

Gross lesions were not observed in rats exposed to 0.0 or 0.07 mg HM/m^3. Fifteen out of twenty of the rats exposed to 0.28 mg HM/m^3 had multiple, soft, white, foci (0.5-1 mm in diameter) disseminated randomly throughout the parenchyma of all the lung lobes. All the rats (20/20) exposed to 1.08 mg HM/m^3 had similar white foci scattered throughout the lung lobes. The number of foci were considerably greater in the lungs of rats exposed to the 1.08 mg HM/m^3 compared to those exposed to 0.28 mg HM/m^3. In addition to the lung lesions in the 1.08 mg HM/m^3 exposed rats, these animals frequently

Table 6

Body Weights and Weight Gain Summary
(Mean ± SD)
Two-Year Repeated Inhalation Exposure of
F344 Rats to HM - Discontinued Study

HM Exposure Concentration (mg/m^3)	Mean Body Weight		Absolute Mean Weight Gain[a] (g)
	Path/tox Day 1	Path/tox Day 175	
Males			
Control	126 ± 11	370 ± 25	244 ± 21
0.08	126 ± 11	360 ± 26	234 ± 21[b]
0.28	122 ± 11	359 ± 22	235 ± 20
1.1	124 ± 11	358 ± 20	234 ± 17[b]
Females			
Control	102 ± 9	206 ± 12	104 ± 11
0.06	101 ± 9	202 ± 10	101 ± 9
0.27	100 ± 9	202 ± 12	102 ± 11
1.0	100 ± 9	197 ± 12	97 ± 10[b]

[a]Absolute mean weight gain determined from the last scheduled in-life weighing referenced to the pre-test body weight.
[b]Analysis of variance indicated that group mean was significantly different from control at $p < 0.05$, using Dunnett's t test.

had enlarged bronchial lymph nodes. This finding was rarely observed in rats exposed to 0.28 mg HM/m^3.

Incidental gross lesions, not related to the HM exposure, were few and inconsequential to the health of these animals.

2. Terminal Body Weights

Terminal body weights were obtained on those rats sacrificed at 6 months (10 rats/group/sex). No dose-related differences in terminal body weights were noted (Tables 7 and 8). These values were used to calculate organ/body weight ratios. Individual terminal body weights and group mean terminal body weights are listed in Appendix D, Table D-3.

3. Organ Weights and Weight Ratios

a. Organ Weights

Mean group organ weights for males and females are given in Tables 7 and 8, respectively. Individual organ weights for males and females are listed in Appendix D, Table D-3. High exposure group male and female rats had statistically increased lung weights compared to control rats. However, there was no significant difference in lung weights of control rats compared to rats exposed to 0.07 or 0.28 mg HM/m^3. The weight of bronchial lymph nodes was statistically higher for female, but not male rats exposed to 1.08 mg HM/m^3.

b. Organ/Body Weight Ratios

Group mean organ/body weight ratios of male and female rats exposed to HM are given in Tables 9 and 10, respectively. Individual values are in Appendix D, Table D-4. Lung/body weight ratios were significantly higher for both male and female rats exposed to 1.08 mg HM/m^3 and were significantly higher ($p < 0.05$) for male rats exposed to 0.28 mg HM/m^3 compared to control rats. The organ/body weight ratio of bronchial

Table 7

6 Month Sacrifice
Terminal Body and Organ Height Summary for Male Rats
(Mean ± SD)
Two-Year Repeated Inhalation Exposures of F344 Rats to HM - Discontinued Study

HM Exposure Concentration (mg/m^3)	Terminal Body Height[a]	Adrenal Glands[a]	Thymus[a]	Lungs[a]	Heart[a]	Liver[a]	Spleen[a]	Testes[b]	Kidneys[b]	Brain[a]	Bronchial Lymph Node[b]
0	345 ± 16	0.081 ± 0.025	0.225 ± 0.033	1.31 ± 0.11	0.921 ± 0.072	8.07 ± 0.44	0.662 ± 0.044	3.17 ± 0.13	2.25 ± 0.25	1.89 ± 0.10	0.010 ± 0.009
0.08	335 ± 29	0.073 ± 0.016	0.219 ± 0.043	1.41 ± 0.17	0.878 ± 0.095	8.44 ± 0.92	0.681 ± 0.074	3.14 ± 0.13	2.32 ± 0.53	1.90 ± 0.06	0.019 ± 0.026
0.28	332 ± 28	0.080 ± 0.016	0.224 ± 0.044	1.41 ± 0.21	0.892 ± 0.129	8.17 ± 1.01	0.668 ± 0.053	3.10 ± 0.33	2.14 ± 0.21	1.90 ± 0.07	0.016 ± 0.019
1.1	341 ± 16	0.067 ± 0.018	0.225 ± 0.051	1.89 ± 0.18[c]	0.931 ± 0.093	8.25 ± 0.72	0.671 ± 0.034	3.16 ± 0.18	2.11 ± 0.12	1.92 ± 0.04	0.017 ± 0.005

[a]Dunnett's t test (data homogeneous by Bartlett's test).
[b]Modified t test (data nonhomogeneous by Bartlett's test).
[c]Group is significantly different from control at $p < 0.01$.

Table 8

6 Month Sacrifice
Terminal Body and Organ Height Summary for Female Rats
(Mean ± SD)
Two-Year Repeated Inhalation Exposures of F344 Rats to HM - Discontinued Study

HM Exposure Concentration (mg/m^3)	Terminal Body Height[a]	Adrenal Glands[a]	Thymus[a]	Lungs[a]	Heart[a]	Liver[a]	Spleen[a]	Ovaries[a]	Kidneys[a]	Brain[a]	Bronchial Lymph Node[b]
0	187 ± 10	0.089 ± 0.036	0.193 ± 0.032	0.95 ± 0.06	0.611 ± 0.045	5.02 ± 0.31	0.430 ± 0.017	0.203 ± 0.117	1.35 ± 0.09	1.78 ± 0.04	0.008 ± 0.005
0.06	185 ± 5	0.096 ± 0.027	0.190 ± 0.034	1.02 ± 0.11	0.615 ± 0.039	4.97 ± 0.28	0.447 ± 0.024	0.161 ± 0.087	1.35 ± 0.07	1.78 ± 0.08	0.007 ± 0.004
0.27	189 ± 9	0.104 ± 0.038	0.183 ± 0.041	1.01 ± 0.07	0.623 ± 0.043	5.19 ± 0.52	0.446 ± 0.027	0.169 ± 0.109	1.38 ± 0.10	1.78 ± 0.05	0.009 ± 0.004
1.0	181 ± 11	0.085 ± 0.017	0.188 ± 0.043	1.45 ± 0.13[c]	0.612 ± 0.033	4.81 ± 0.37	0.457 ± 0.039	0.189 ± 0.118	1.37 ± 0.09	1.74 ± 0.07	0.020 ± 0.008[c]

[a]Dunnett's t test (data homoegeneous by Bartlett's test).
[b]Modified t test (data nonhomogeneous by Bartlett's test).
[c]Group is significantly different from control at $p < 0.01$.

Table 9

6 Month Sacrifice
Percent Organ-to-Body Height Ratio for Male Rats
(Mean ± SD)
Two-Year Repeated Inhalation Exposures of F344 Rats to HM - Discontinued Study

HM Exposure Concentration (mg/m^3)	Adrenal Glands[a]	Thymus[a]	Lungs[a]	Heart[a]	Liver[b]	Spleen[a]	Testes[a]	Kidneys[b]	Brain[a]	Bronchial Lymph Node[b]
0	0.023 ± 0.007	0.065 ± 0.008	0.379 ± 0.022	0.267 ± 0.024	2.34 ± 0.09	0.192 ± 0.011	0.920 ± 0.041	0.652 ± 0.064	0.547 ± 0.028	0.003 ± 0.003
0.08	0.022 ± 0.006	0.065 ± 0.012	0.420 ± 0.040	0.262 ± 0.014	2.52 ± 0.14[c]	0.204 ± 0.023	0.941 ± 0.054	0.693 ± 0.155	0.569 ± 0.036	0.006 ± 0.008
0.28	0.024 ± 0.004	0.067 ± 0.011	0.423 ± 0.045[d]	0.268 ± 0.018	2.46 ± 0.23	0.202 ± 0.012	0.932 ± 0.069	0.644 ± 0.044	0.576 ± 0.048	0.005 ± 0.005
1.1	0.019 ± 0.005	0.066 ± 0.014	0.554 ± 0.038[c]	0.273 ± 0.028	2.41 ± 0.11	0.197 ± 0.010	0.928 ± 0.052	0.617 ± 0.018	0.565 ± 0.025	0.005 ± 0.001

[a]Dunnett's t test (data homogeneous by Bartlett's test).
[b]Modified t test (data nonhomogeneous by Bartlett's test).
[c]Group is significantly different from control at $p < 0.01$.
[d]Group is significantly different from control at $p < 0.05$.

Table 10

6 Month Sacrifice
Percent Organ-to-Body Height Ratio for Female Rats
(Mean ± SD)
Two-Year Repeated Inhalation Exposures of F344 Rats to HM - Discontinued Study

HM Exposure Concentration (mg/m^3)	Adrenal Glands[a]	Thymus[a]	Lungs[a]	Heart[b]	Liver[b]	Spleen[a]	Ovaries[a]	Kidneys[a]	Brain[a]	Bronchial Lymph Node[a]
0	0.048 ± 0.020	0.103 ± 0.016	0.510 ± 0.037	0.326 ± 0.017	2.68 ± 0.08	0.230 ± 0.008	0.108 ± 0.062	0.724 ± 0.052	0.953 ± 0.053	0.004 ± 0.002
0.05	0.052 ± 0.015	0.103 ± 0.019	0.552 ± 0.058	0.333 ± 0.023	2.69 ± 0.14	0.242 ± 0.015	0.082 ± 0.046	0.731 ± 0.042	0.961 ± 0.043	0.004 ± 0.002
0.27	0.055 ± 0.020	0.097 ± 0.019	0.537 ± 0.048	0.331 ± 0.024	2.75 ± 0.25	0.237 ± 0.021	0.089 ± 0.057	0.735 ± 0.054	0.946 ± 0.041	0.005 ± 0.002
1.0	0.047 ± 0.010	0.103 ± 0.021	0.602 ± 0.078[c]	0.339 ± 0.008	2.66 ± 0.14	0.253 ± 0.018[c]	0.105 ± 0.066	0.758 ± 0.042	0.964 ± 0.067	0.011 ± 0.002[c]

[a]Dunnett's t test (data homogeneous by Bartlett's test).
[b]Modified t test (data nonhomogeneous by Bartlett's test).
[c]Group is significantly different from control at $p < 0.01$.

lymph nodes from female rats exposed to 1.08 mg HM/m^3 was significantly increased compared to controls. The organ/body weight ratio of spleen was significantly elevated above control in female rats exposed to 1.08 mg HM/m^3. This increase is probably not of biological significance since the actual mean spleen weight is not statistically significant from controls (Table 8) and is a result of a slight increase in spleen weight (Table 8) along with a slight decrease in mean body weight (Table 8). The statistically significant increase in the liver/body weight ratio for male rats exposed to 0.07 mg HM/m^3 is probably of no biological significance since the effect is slight and is not seen at higher doses and is not reflected in the organ/brain weight ratio (Table 11) or in the mean liver weight (Table 8).

c. Organ/Brain Ratios

Group mean organ/brain weight ratios of male and female rats exposed to HM are given in Tables 11 and 12, respectively. Individual values are in Appendix D, Table D-5. Lung/brain weight ratios of male and female rats were significantly higher than controls in the 1.08 mg HM/m^3 exposure group. The spleen/brain weight ratio was statistically higher ($p < 0.05$) in female rats, however, the increase was slight (< 5%) and probably has little biological significance. The bronchial lymph node/brain weight ratio was significantly increased in female rats exposed to 1.08 mg HM/m^3.

G. Histopathology

Rats were exposed for approximately 6 months to 0, 0.07, 0.28, or 1.08 mg HM/m^3. From each rat sacrificed, one tissue section from the left diaphragmatic lung lobe, the right apical lung lobe, the right cardiac lung lobe, and the right diaphragmatic lung lobe were microscopically examined. In

Table 11

6 Month Sacrifice
Percent Organ-to-Brain Height Ratio for Male Rats
(Mean ± SD)
Two-Year Repeated Inhalation Exposures of F344 Rats to HM - Discontinued Study

HM Exposure Concentration (mg/m^3)	Adrenal Glands[a]	Thymus[a]	Lungs[a]	Heart[a]	Liver[a]	Spleen[a]	Testes[b]	Kidneys[b]	Bronchial Lymph Node[b]
0	4.28 ± 1.34	11.93 ± 1.78	69.47 ± 6.01	48.96 ± 5.09	428.77 ± 34.21	35.16 ± 2.70	168.48 ± 11.97	119.47 ± 13.94	0.534 ± 0.497
0.08	3.85 ± 0.88	11.54 ± 2.22	74.05 ± 8.12	46.21 ± 4 ;	444.09 ± 37.51	35.90 ± 3.92	165.54 ± 5.80	121.93 ± 25.92	0.993 ± 1.359
0.28	4.20 ± 0.77	11.73 ± 2.14	73.81 ± 10.26	46.90 ± 6.70	428.73 ± 46.44	35.17 ± 3.21	162.63 ± 16.71	112.35 ± 9.90	0.827 ± 0.964
1.1	3.46 ± 0.91	11.68 ± 2.62	98.33 ± 9.33[c]	48.48 ± 5.43	428.96 ± 34.73	34.87 ± 1.93	164.41 ± 8.45	109.50 ± 5.74	0.886 ± 0.265

[a]Dunnett's t test (data homogeneous by Bartlett's test).
[b]Modified t test (data nonhomogeneous by Bartlett's test).
[c]Group is significantly different from control at $p < 0.01$.

Table 12

6 Month Sacrifice
Percent Organ-to-Brain Height Ratio for Female Rats
(Mean ± SD)
Two-Year Repeated Inhalation Exposures of F344 Rats to HM - Discontinued Study

HM Exposure Concentration (mg/m^3)	Adrenal Glands[a]	Thymus[a]	Lungs[b]	Heart[a]	Liver[a]	Spleen[a]	Ovaries[a]	Kidneys[a]	Bronchial Lymph Node[a]
0	5.02 ± 2.10	10.84 ± 1.89	53.50 ± 3.03	34.35 ± 2.70	282.04 ± 20.28	24.15 ± 1.09	11.46 ± 6.77	76.06 ± 5.19	0.446 ± 0.276
0.06	5.39 ± 1.45	10.74 ± 1.94	57.44 ± 5.62	34.65 ± 1.27	280.50 ± 17.02	25.26 ± 2.38	8.53 ± 4.92	76.14 ± 4.80	0.394 ± 0.228
0.27	5.85 ± 2.20	10.25 ± 2.18	56.78 ± 4.36	34.98 ± 2.53	291.41 ± 29.72	25.08 ± 1.82	9.55 ± 6.34	77.77 ± 6.38	0.481 ± 0.262
1.0	4.90 ± 1.05	10.84 ± 2.73	83.45 ± 8.99[c]	35.24 ± 2.62	277.33 ± 24.89	26.34 ± 2.27[d]	10.86 ± 6.61	78.76 ± 4.76	1.14 ± 0.44[c]

[a]Dunnett's t test (data homogeneous by Bartlett's test).
[b]Modified t test (data nonhomogeneous by Bartlett's test).
[c]Group is significantly different from control at $p < 0.01$.
[d]Group is significantly different from control at $p < 0.05$.

addition, one tissue section from a bronchial lymph node from each rat was also examined.

1. Histopathological Definitions

The term alveolitis in this study was defined as an HM exposure-induced increase in the numbers of neutrophils within the alveolar septa and airspaces in the centriacinar regions of the lung (i.e., alveolar ducts and proximal alveoli).

The term macrophage hyperplasia was defined as an HM exposure-induced increase in the number of alveolar macrophages within the centriacinar region and/or proximal alveoli of the lung. Hyperplastic macrophages were often in aggregates of 3-15 and moderately hypertrophic with foamy cytoplasm and ovoid vesicular nuclei. Some of these phagocytic cells had pyknotic nuclei and/or phagocytic cellular debris within their cytoplasms.

Epithelial hyperplasia within the lung was defined as an HM exposure-induced increase in the number of type II epithelial cells lining the alveolar ductal and/or proximal alveolar airspaces (i.e., centriaci :r region of the lung).

All of the lung lesions defined above were induced by HM exposure and each lesion was closely associated with the other two lesions in the centriacinar regions of the lung. These histologic alterations correlated with the disseminated white foci observed grossly in the lungs of rats necropsied after exposure to 0.28 mg HM/m^3 and 1.08 mg HM/m^3.

Lymphoid hyperplasia of the bronchial lymph nodes or the bronchial-associated lymphoid tissue (BALT) was defined as an increased number of lymphoid cells within the extrapulmonary lymph node or within the intrapulmonary bronchial airway-associated lymphoid tissue, respectively.

Severity of the pulmonary lesions (i.e., alveolitis, macrophage hyperplasia, epithelial hyperplasia, and lymphoid hyperplasia of BALT) were characterized as minimal (i.e., less than 10% of the lung section affected); mild (i.e., more than 10%, but less than 25% of the lung section was affected); moderate (i.e., more than 25%, but less than 50% of the lung section was affected); or marked (i.e., more than 50% of the lung was affected).

The severity of lymphoid hyperplasia of bronchial lymph nodes was characterized as minimal when approximately 1.5 times more lymphoid cells were present in lymph nodes than in air controls; mild when approximately twice as many lymphoid cells were present in the lymph node than in air controls; moderate when approximately 3 times more lymphoid cells were present in the node than in air controls; or marked when approximately 4 times more lymphoid cells were present in the lymph nodes than in air controls.

2. Pulmonary Findings in Control and 0.07 mg HM/m^3-Exposed Rats

Rats in the air control and 0.07 mg HM/m^3-exposed groups had no microscopic lesions in any of the lung sections examined.

3. Pulmonary Findings in 0.28 mg HM/m^3-Exposed Rats

In the 0.28 mg HM/m^3 exposure group, 80% of the male and female rats had alveolitis and type II epithelial cell hyperplasia associated with alveolar macrophage hyperplasia in the centriacinar regions of the lung. Neutrophils were present both in centriacinar airspaces and in the septa of alveolar ducts and proximal alveoli. Proteinaceous and cellular debris was also present in some of the airspaces. The severity of the alveolitis, macrophage hyperplasia, and the proliferation of pneumocytes in these rats were, however, minimal.

Minimal lymphoid hyperplasia of BALT was only present in the lung sections of 30% of the females and 20% of the males in the 0.28 mg HM/m^3 exposure group.

4. Pulmonary Findings in 1.08 mg HM/m^3-Exposed Rats

The most severe pulmonary lesions associated with the HM exposure were evident in the rats from the highest exposure level group (i.e., 1.08 mg HM/m^3). The character of the centriacinar lesions of alveolitis, macrophage hyperplasia, and type II epithelial hyperplasia, and the bronchial changes of lymphoid hyperplasia of BALT were similar to those in the rats from the 0.28 mg HM/m^3 exposure group. The principal differences in the lung lesions between the two groups were in the incidences and severity of the lesions. More rats (10/10 M, 10/10 F) in the 1.08 mg HM/m^3 exposure group had lung lesions compared to the 0.28 mg HM/m^3-exposed rats (8/10 M, 8/10 F), and these lesions were more severe than in rats exposed to 0.28 mg HM/m^3. Males in the highest exposure group had moderate macrophage hyperplasia that was associated with moderate type II epithelial cell hyperplasia and mild alveolitis in the centriacinar alveolar ducts and proximal alveoli. In addition, these rats had mild lymphoid hyperplasia of BALT. Female rats in the highest exposure group had moderate macrophage hyperplasia in the centriacinar alveolar ducts and proximal alveoli, along with mild centriacinar alveolitis and associated, mild, type II epithelial cell hyperplasia. Female rats in this exposure group also had minimal lymphoid hyperplasia of BALT.

5. Bronchial Lymph Node Findings

No microscopic lesions were evident in any of the lymph node sections examined from rats in the control and the 0.07 mg HM/m^3-exposed group. One male and 4 females in the 0.28 mg HM/m^3 exposure group had minimal

to mild lymphoid hyperplasia in the paracortical region of the bronchial lymph node. Mild to marked lymphoid hyperplasia was evident in 100% of the males and 90% of the females exposed to 1.08 mg HM/m^3 of HM.

6. Summary Tables

An individual animal summary of microscopic lesions of rats with pulmonary and/or bronchial lymph node lesions is given in Appendix E.

IV. DISCUSSION

It was planned to expose F344 rats to aerosols of HM at concentrations of 0, 0.05, 0.2, and 0.8 mg/m^3 for 6 hr/day, 5 days/week for 2 years. However, just before the 6-month interim sacrifice period, it was discovered that exposure chamber sampling procedures to determine aerosol concentrations were done incorrectly. This resulted in an underestimation of the actual exposure concentrations by approximately 38%. Because of this, P&G decided to end the current study after the 6-month sacrifices and restart these chronic inhalation studies.

During the 6 months of exposures, no mortality occurred in either male or female rats and no clinical signs of toxicity were seen during this period for both sexes. Decreases in body weights for both male and female rats were slight and variable with no clear dose-response relationship. At necropsy, exposure-related gross pathological lesions were limited to multiple, soft, white foci in all lung lobes and enlarged bronchial lymph nodes in rats exposed to the mid and high exposure levels. Exposure-related changes in organ weights were confined to increases in lung weights, lung/body weight ratios, lung/brain weight ratios for both sexes and increases in the bronchial lymph node weight, bronchial lymph node/body weight and bronchial lymph

node/brain weight ratios of female rats exposed to 1.08 mg HM/m^3. Histopathological evaluations of the lungs and bronchial lymph nodes showed pulmonary lesions characterized by centriacinar alveolitis, macrophage hyperplasia, type II epithelial cell hyperplasia, and lymphoid hyperplasia of bronchial associated lymphoid tissue, respectively, and only in male and female rats exposed to 0.28 and 1.08 mg HM/m^3.

Since the discovery of the sampling error, exposure laboratory procedural changes have been made and documented so that such errors will not occur again. As directed by P&G, the ITRI will reinitiate the 2-year repeated inhalation exposure study of HM (W2004.02) in F344 rats (ITRI Protocol FY90-010). The results from this new chronic study will be described in a separate report.

STUDY INVESTIGATORS

J. D. Sun, PhD — Date 12/17/90
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P. J. Sabourin, PhD — Date 12/17/90
Toxicologist

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C. H. Hobbs, DVM — Date 12/17/90
Assistant Director

J. L. Mauderly, DVM — Date 12/17/90
Director

V. QUALITY ASSURANCE STATEMENT

TEST CHEMICAL: HM - W1433.02

STUDY TYPE: ITRI Protocol FY89-009: Two-Year Repeated Inhalation Exposure of F344/N Rats to HM - Discontinued Study

This study was inspected by the Inhalation Toxicology Research Institute Quality Assurance Unit. Findings were discussed with the study scientists at time of inspection and written reports were submitted to the study director and to management.

QA UNIT INSPECTION SCHEDULE

Study Phase	Inspection Date	Report Date
Protocol Audit:		
Audit	13-MAR-89	13-MAR-89
Approval	23-MAR-89	23-MAR-89
Approval Path/Tox	15-MAR-89	15-MAR-89
	03-APR-89	03-APR-89
Prestudy Inspections:		
Animal Receipt	07-MAR-89	15-MAR-89
Body Wt/Clin Obs	23-MAR-89	10-APR-89
Test Chemical	23-MAR-89	10-APR-89
Exposure Room	23-MAR-89	10-APR-89
Animal ID	07-APR-89	10-APR-89
Conditioning Rm Records	10-APR-89	10-APR-89
In-Life Inspections:		
Dose Prep/Administration	10-APR-89	10-APR-89
	20-JUN-89	20-JUN-89
	18-AUG-89	18-AUG-89
Chemistry Laboratory	05-MAY-89	05-MAY-89
	23-AUG-89	23-AUG-89
Body Wt/Clin Obs	06-MAY-89	06-MAY-89
	10-JUN-89	11-JUN-89
Necropsy	25-SEP-89	25-SEP-89

QA UNIT INSPECTION SCHEDULE (CONT.)

Study Phase	Inspection Date	Report Date
Data Audits:		
Exposure Data	17-APR-89	17-APR-89
	16-JUN-89	16-JUN-89
	25-AUG-89	25-AUG-89
	25-OCT-89	26-OCT-89
	11-JUN-90	11-JUN-90
Prestudy Data	21-APR-89	24-APR-89
Body Wt/Obs Data	24-APR-89	25-APR-89
	07-JUN-89	07-JUN-89
	28-AUG-89	29-AUG-89
Chemistry Data	16-JUN-89	16-JUN-89
All Data	05/08-JUN-90	08-JUN-90
Final Report Audit:		
Draft Report	05/08-JUN-90	08-JUN-90
	03-JUL-90	03-JUL-90
	30-JUL-90	30-JUL-90
Final Report	27-NOV-90	27-NOV-90

D. L. Harris 12-17-90
D. L. Harris Date
QA Officer

APPENDIX A & B — on file

TWO-YEAR REPEATED INHALATION EXPOSURE OF F344 RATS TO HM - DISCONTINUED STUDY

FINAL REPORT

DATE: December, 1990

Submitted to: Dr. Robert C. Lindenschmidt, Divisional Toxicologist
The Procter and Gamble Company
Winton Hill Technical Center
6100 Center Hill Road
Cincinnati, OH 45224-1788

Submitted by: Inhalation Toxicology Research Institute
Lovelace Biomedical and Environmental Research Institute
P. O. Box 5890
Albuquerque, NM 87185

Study Director: Dr. James D. Sun

ITRI Study No.: FY89-009
TSIN: M1433.02
DRD No.: PDSE 957

Prepared for The Procter and Gamble Company under Funds-In-Agreement Number DE-F104-86AL39741 with the Lovelace Inhalation Toxicology Research Institute, which is operated for the U. S. Department of Energy under DOE Contract Number DE-AC04-76EV01013.

LD

APPENDIX C, D, & E – on file

TWO-YEAR REPEATED INHALATION EXPOSURE OF F344 RATS TO HM – DISCONTINUED STUDY

FINAL REPORT

DATE: December, 1990

Submitted to: Dr. Robert C. Lindenschmidt, Divisional Toxicologist
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Submitted by: Inhalation Toxicology Research Institute
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Study Director: Dr. James D. Sun

ITRI Study No.: FY89-009
TSIN: M1433.02
DRD No.: PDSE 957

Prepared for The Procter and Gamble Company under Funds-In-Agreement Number DE-F104-86AL39741 with the Lovelace Inhalation Toxicology Research Institute, which is operated for the U. S. Department of Energy under DOE Contract Number DE-AC04-76EV01013.

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